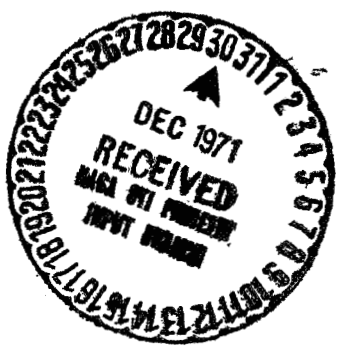
CR 115286

Final Report
NASA Contract, NAS9-11642

Histochemical and Biochemical Analyses of Plant Tissues
Robert S. Halliwell, Project Leader

A certificate of satisfactory completion of this contract issued by the technical monitor, Dr. Charles H. Walkinshaw is appended.

This report summarizes the work carried out by the contractor and lists the experiments of which the raw data was submitted to the technical monitor. Results and conclusions can not be drawn by the contractor as the specimens analyzed were only identified by number, the treatments were not known.



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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER
HOUSTON, TEXAS 77058

REPLY TO
ATTN OF: DC52-L152-71

NOV 2 7 1971

R. S. Halliwell, Ph. D.
Department of Plant Sciences
Texas A&M University
College Station, TX 77843

Dear Doctor Halliwell:

This is to certify that I am in receipt of all of the necessary rough notes, experimental results, histological specimens, and photographs required by the terms of the NASA Contract, NAS9-11642. All materials received have been satisfactory. Your performance on this contract is to be commended.

Your final report, which is due December 1, 1971, should summarize the highlights of progress under the terms of the contract. All publications prepared by my staff using any of the data generated by this contract will acknowledge by authorship or footnotes the contributions made by you and your staff to the successful follow-on tests from the Apollo 14 post-flight quarantine of lunar material.

Sincerely,

Charles H. Walkinshaw

Charles H. Walkinshaw, Ph. D.
Technical Monitor, NAS9-11642
Preventive Medicine Division

Analyses Performed on Apollo-14 Exposed Tissue Cultures

Samples consisted of cell homogenates prepared from a number of tissue cultures employed in the Apollo 14 quarantine. Cultures had been grown without treatment and with treatment by incorporation of measured quantities of lunar fines or finely divided terrestrial basalt into the agar medium. Plant tissues employed in the experiment included, longleaf pine, tobacco, soybean, rice, carrot, sunflower, okra, lettuce, and spurge. All tissues, a total of 95 samples, were subjected to the following analyses:

1. Total nitrogen- Samples were digested by the kjeldahl method and quantitated by the phenol-hypochlorite color reaction.

2. Total phoshorus- Samples were digested with H_2SO_4 hydrogen peroxide and quantitated by the Fiske-Subbarow method.

3. Protein- a). Soluble protein was separated from cellular particles by centrifugation and then precipitated with trichloroacetic acid. b). Total protein was separated from small molecules by methanol extraction and then dissolved in sodium hydroxide (1 N).

   Quantitation of soluble and total protein was by the Lowry method.

4. Soluble phenolics- Samples were extracted with methanol. Total phenolics in the methanol extracts were quantitated by the Folin phenol method. Comparison was with a resorcinol standard.

5. Nucleic Acids- Samples were extracted by the hot NaCl method.

   Analysis was by:
   a). Absorbance ratios 280/260 of Warburg and Christian for estimation of total nucleic acids.
   b). The Orcinol method for estimation of RNA
   c). The Diphenylamine method for estimation of DNA

6. Total non-specific acid phosphatase- Total cell homogenates were assayed for enzyme activity by incubation with -Glycero-phosphate at pH 5.0 in the presence of Triton X-100.

In addition to the analyses listed above selected sets of tissues were subjected to additional analyses:

1. A set of 15 additional pine culture homogenates was analyzed in the same manner as the original samples. These homogenates had been prepared in distilled water rather than in sucrose-Tris buffer as was the case for all other tissues. These samples were analyzed, in addition, for total soluble carbohydrate.

2. Three sets of tissues, lettuce, longleaf pine, and tobacco, (30 samples of the original 95) were serially extracted with:

   a). Hot 70% ethanol. These extracts were sent to the Manned Spacecraft Center to be used for Amino acid analysis.
   b). Perchloric acid (26%) at room temperature. These extracts were used to estimate starch content of the samples by the Anthrone reaction.
   c). NaOH (1N). Total protein in these extracts was estimated by the Lowry method.

3. Separate samples from all of the above sets of tissues were extracted with cold 5% Trichloroacetic acid. These extracts were sent to the Manned Spacecraft Center for measurement of ATP.

Histological Studies:

Tissue specimens were embedded in Epoxy resin and;

1. Sectioned, stained with preferential stains, examined with a light microscope, photomicrographs of the tissue sections were taken and submitted to the technical monitor.

2. Additional embedded specimens were given to the technical monitor for electron microscopy studies.

Respiration Studies:

Respiration studies were completed on the following plant specimens each including lunar challenged and appropriate controls.

1. Plant Tissue included- Carrot, soybean, lettuce, sunflower, pine, rice, cotton, corn, okra, tobacco, and Euphoria.

2. Cultured plant cells included pine and tobacco.

Purchases Totaling Over $100.00

1.	*Glassware for Gilson Respirometer (Gilson Medical Electronics)	$334.00
2.	*Cell Holder-accessory for Beckman Spectrometer (Beckman Co.)	$174.25
3.	*Pipets (micro) (Brinkmann Inst. Co.)	$199.50
4.	Rotary Shakers (2 ea. @ $269.00) (New Brunswick Scientific)	$538.00
5.	*Centrifuge Tubes (A. H. Thomas Co.)	$154.00
6.	*Film and Prepaid Mailers (So. West Photo Co.)	$145.00
7.	*Glassware and Chemicals (TAMU Biochemistry Supply)	$140.26

It is requested at this time that the non-expendable item No. 4 (Rotary Shaker) be retained by the contractor until the termination of NASA contract NAS 9-12050.

*Expendable